120 Epidemiology and identification of non-lactose fermenter bacteria in an adult cystic fibrosis centre

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Introduction: St. Vincent's University Hospital caters for approximately 300 patients in its adult CF centre. Over 70% of these patients are colonized with *P. aeruginosa*. Other organisms commonly isolated are: *S. aureus*, *S. maltophilia* and *B. cepacia* complex. These organisms can usually be identified by conventional methods such as API20NE.
Some less common organisms can be more difficult to identify and are less likely to grow on media such as Maconky agar. In SVUH, we have recently begun to use 16S sequencing on difficult to identify NLFs.
Method: In 2010, 24 isolates were identified by 16S sequencing.
Results: See the table.

Table 1

Organism	Number
Bordetella petrii	1
Pandoraea spp.	2
Chryseobacterium spp.	7
A. xylosoxidans	7
Sphingobacterium spiritivorans	5
Delftia tsuruhatenis	1
Burkolderia gladioli	1

All isolates were sent to HPA Colindale to confirm results.
Conclusion: This study has shown that organisms more commonly known as colonizers of CF patients such as *A. xylosoxidans* and *Pandoraea spp* can prove difficult to identify by conventional means. We hope to develop a PCR assay specific for *A. xylosoxidans*.
Chryseobacterium spp and *Sphingobacterium spp* are becoming increasingly common in our CF patients but the significance of these organisms is still unknown.

121 Identification of biochemically inert non-fermentative bacteria by MALDI-TOF MS

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Sputa of cystic fibrosis (CF) patients often contain inert non-fermentative bacilli rendering standard tests useless for identification. Our reference laboratory applies costly and time-consuming biochemical tests, fatty acid analysis and 16S sequencing for identification. MALDI-TOF MS can reliably identify mucoid non-inert non-fermentative bacilli, but its performance on inert strains is unknown. We compared MALDI-TOF MS with reference identification tests for these bacilli. All biochemical inert non-fermentative bacteria from CF sputa collected between 01–2007 and 06–2010, that were identified by the reference laboratory, were investigated by MALD-TOF MS. Biochemical inert was defined as DNAse, acetamide and lysine test negative after 24 hours and no growth on C390/phenatroline agar. Of 78 selected isolates, 17 were unavailable for analysis and 4 were duplicates. Compared to reference methods, 52 (91%) isolates had correct genus identification and 5 (9%) were unidentifiable; none had incorrect genus identification. Fifty isolates had species identification, of which 41 (82%) were correctly identified by MALDI-TOF MS, 5 (10%) had no species identification and 4 (8%) had incorrect species: *P. monteilli* vs. *P. putida* (3x), *A. ruhlandii* vs. *A. xylosoxidans*. Note that *P. monteilli* belongs to the *P. putida* group. All 11 *Burkholderia sp.* isolates were correctly identified to *B. cepacia* complex, but within the complex 2 isolates were incorrectly identified: *B. stabilis* vs. *B. cenocepacia*, *B. multivorans* vs. *B. cepacia*. We conclude that MALDI-TOF MS can identify inert non-fermentative bacilli, although some strains can only be identified to *P. putida* group or *B. cepacia* complex.

122 A one-year experience of routine identification by MALDI-TOF MS of non-fermenting Gram-negative rods recovered from respiratory samples from cystic fibrosis patients

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Correct identification of non-fermenting gram-negative bacteria (NFGNB) recovered from respiratory samples of cystic fibrosis (CF) patients by conventional microbiology methods is often difficult.
Objective: To describe our one-year experience (2010) using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MT) as the routine identification method for NFGNB recovered from CF patients.
Methods: Data were collected by analysis of database case records of 122 adult and paediatric patients attending at the CF Unit in 2010. A total of 80 NFGNB isolates (excluding *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia)* were obtained during routine follow-up visits from 44 CF patients. For the identification, MT examines the profile of proteins detected directly from intact bacteria, using FlexControl 3.0 and MALDI BioTyper 2.0 (Bruker Daltonik, Germany).
Results: All strains, except one (n = 79) displayed identification using MT. 32 *Achromobacter xylosoxidans*, 11 *Chryseobacterium indologenes*, 9 *Burkholderia cepacia* complex, 9 non-*aeruginosa Pseudomonas*, 5 *Elizabethkingia meningoseptica* 3 *Bordetella bronchiseptica,* 2 *Agrobacter tumefaciens,* 2 *Ochrobactrum anthropi,* 2 *Pandoraea* sp., 2 *Sphingobacterium* sp., 1 *Acinetobacter* sp. and 1 *Alcaligenes* sp. were detected. Lack of identification (1.2%) was ascribed to insufficient database entries. MT has proven to distinguish the closely related *Burkholderia multivorans* (n = 5) and *B. cepacia* (n = 4). Additionally, *Pandoraea pnomenusa* (n = 2), not identified by phenotypical methods, were correctly identified by MT.
Conclusions: MT is a versatile tool that improves rapid identification of bacteria including those recovered from CF patients with limited biochemical reactivity frequently misidentified by the classical approach.

123 Identification of the main cystic fibrosis pulmonary pathogens by SNuPE (single nucleotide primer extension)

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Respiratory infections remain a major threat to Cystic Fibrosis (CF) patients. The detection and correct identification of the bacteria responsible for these infections is critical for the therapeutic management of patients. The traditional methods of culture and phenotypic identification of bacteria lack both sensitivity and specificity because many bacteria can be missed and/or misidentified. To overcome this obstacle, a plethora of different molecular analyses have been set up in the last years.
The aim of this study was to set up a diagnostic method based on SNuPE (Single Nucleotide Primer Extension) for the detection of the main CF pulmonary pathogens, which has been recently used for the discrimination of bacteria belonging to different species. This technique allows the detection of the nucleotide located at any site of a DNA sequence. We focused our attention on 16S rRNA gene, which is the most commonly used marker of microbial diversity. A total of 2469 16S rDNA sequences, representative of 12 of the most common CF pathogens, were analyzed for the presence of both highly conserved and polymorphic regions. This analysis allowed to design a set of primers of different length for the SNuPE technique, specific for four pathogens: *Pseudomonas aeruginosa, Staphylococcus aureus, Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex bacteria. These primers were used either in simplex or multiplex SNuPE reactions using the DNA of the four pathogens. Data obtained revealed that the SNuPE profiles allowed the easy identification of isolates belonging to the four species. The set up of the system directly on CF patients sputum is in progress.